pancreatic ductal epithelial cells, a mechanical profile that was partially corrected with 4-HAP. Tests of 4-HAP in mouse models of metastatic pancreatic disease are underway. Overall, 4-HAP modifies nonmuscle myosin II-based cell mechanics across phyla and disease states and provides proof-of-concept that cell mechanics offer a rich drug target space, allowing for possible corrective modulation of tumor cell behavior.

711-Pos Board B491
SH3 Domain of C-Src Regulates its Dynamic Behavior in the Cell Membrane
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Src family kinases are major non-receptor tyrosine kinase in cells and Src-mediated signal transduction involves various cellular functions. In activation process, Src molecules translocate to cell membrane and the subdomains in Src, SH2 and SH3 domains, are exposed. For Src to serve as a kinase, the interaction with its substrates via SH2 and/or SH3 domains is required. Although the activation mechanism of Src has been well-studied, the dynamics of Src at the cell membrane is still unclear. In this study, we examined the role of Src subdomains, especially SH2 and SH3 domains, on the dynamics of Src at the cell membrane. To achieve this, we constructed PAmCherry-tagged wild-type Src (SrcWT), SrcW121A and SrcR178A mutants that decrease the binding of Src to its substrate(s) via SH3 and SH2 domains, respectively, and traced individual Src molecules in the cell membrane with gentle activation of PAmCherry. SrcWT dynamically moved on the cell membrane in the range of 0.27 ± 0.01 μm2/s within a few seconds. The dynamics of SrcR178A mutant was comparable with that of SrcWT, whereas SrcW121A mutant exhibited less mobility (0.16 ± 0.01 μm2/s) at the cell membrane compared with SrcWT. Since both SrcW121A and SrcR178A mutants showed higher phosphorylation level than SrcWT, the result indicates that the less mobility of SrcW121A in the cell membrane seems not to depend upon Src activation status. We further demonstrate that SrcW121A mutant showed ~30% increase in the Src molecules residence time at focal adhesion compared with SrcWT, which is mediated by slower dissociation from adhesion site. Taken together with enhanced localization of SrcW121A at focal adhesion, our findings show that the SH3 domain of Src molecules governs dynamics of Src at the cell membrane, which may be involved in the rapid signal transduction in cells.

712-Pos Board B492
Insights on RGD-Based Peptide Interactions with Integrin Receptors from Atomistic Simulations
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Here we develop a method for generating atomistically derived input parameters for a multiscale methodology to predict surface adhesion for functionalized nanocarriers. This methodology involves two primary steps: (i) AutoDock for determining the ligand-binding pocket and equilibrium-binding energy for receptor-peptide interaction, and (ii) an atomistic PMF calculation methodology for deriving the force vs. distance curves which serves as an input for the multiscale model. We also share some fundamental understanding of the differences in binding behavior due to changes in peptide sequences, especially the residues flanking the key binding mediator: the RGD. These differences in behavior at the binding pocket can be leveraged to better design the functional peptides to enable desired binding of cells to surfaces.

713-Pos Board B493
h2-calponin Gene Knockout Increases Traction Force of Mouse Fibroblasts in vitro
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Cell traction force (CTF) plays a critical role in controlling cell shape, enabling cell motility, and maintaining cellular homeostasis during various biological processes such as wound healing, angiogenesis, and cancer metastasis. It has been demonstrated that h2-calponin, an actin binding protein found in smooth muscle and non-muscle cells including epithelial cells, endothelial cells, macrophages and fibroblasts, plays a role in regulating the actin cytoskeleton activities in cell adhesion, migration and cytokinesis. We recently found that knockout (KO) of h2-calponin gene increased cell motility when compared to wild-type (WT) cells. This finding indicated a potential involvement of h2-calponin in producing CTF. The present study investigated the role of h2-calponin in mouse fibroblast traction force. Primary fibroblasts were isolated from leg muscles of h2-calponin KO and WT mice and analyzed using CTF-microscopy. CTF-microscopy is the current state-of-the-art method to determine CTF in a cell spread on a two-dimensional substrate. Using CTF-microscopy, we determined the root-mean square traction force, the total strain energy, net contractile movement produced by mouse fibroblasts cultured on a thin layer of 8-kPa polyacrylamide gel containing fluorescent beads of 0.2 μm in diameter. The results showed that h2-calponin KO fibroblasts had greater traction force than WT control. In comparison to WT cells expressing abundant tropomyosin-2, h2-calponin KO fibroblasts lost tropomyosin-2, a phenotype mimicking that of metastatic cancer cells. H2-calponin KO fibroblasts also adhered to cultural substrate slower than WT control, had smaller cell spreading area, and rounded up faster during trypsin treatment, supporting the role of h2-calponin in stabilizing the actin cytoskeleton. Our findings indicate that h2-calponin has an inhibitory role in the production of CTF, consistent with the increased motility of h2-calponin-null cells. Further studies on the mechanisms of h2-calponin-mediated CTF regulation and cell motility are underway.

714-Pos Board B494
Deletion of h2-calponin in Macrophages Facilitates Cell Motility and Lipid Clearance: A Novel Mechanism to Attenuate Arterial Atherosclerosis
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Arterial atherosclerosis, a major cardiovascular risk condition, is a chronic inflammatory disease. The atherosclerotic plaques are built up by excessive lipid deposition and accumulation of apoptotic immune cells. Macrophages migrate into atherosclerotic lesions and function in scavenging extracellular lipid and mediating the inflammatory process. Lipid-laden macrophages transform into foam cells and loss their migration ability. The retention of foam cells at arterial intima promotes the growth of atherosclerotic plaques. Calponin is an actin filament-associated protein and its h2 isoform regulates cell proliferation, migration and other cell motility-based functions. We previously demonstrated that removal of h2-calponin in macrophages enhances cell migration and phagocytosis. Deletion of h2-calponin in macrophages significantly attenuated the development of inflammatory arthritis in mouse models (our unpublished results). In the present study, we investigated the function of h2-calponin-null mouse macrophages and foam cells in lipid clearance as well as their migration and transendothelial migration abilities. Foam cells are produced in culture by loading mouse peritoneal macrophages with acetylated low density lipoprotein. Lipid phagocytosis was quantified using Oil Red O staining of intracellular lipid droplets. Migration and transendothelial migration were examined using Transwell assay system. Foam cell apoptosis was studied using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The results showed that h2-calponin-null macrophages exhibit significantly higher lipid engulfment and faster migration and transendothelial migration than wild type controls. The h2-calponin-null foam cells retained higher migration capacity than that of wild type cells, which potentially facilitates migrating out of the arterial intima, reducing accumulation of apoptotic cells, and attenuating atherosclerotic lesions. The data demonstrate that h2-calponin is a novel molecular target for modulating macrophage functions and the development of new therapeutic approaches to the prevention and treatment of atherosclerosis.

Membrane Pumps, Transporters, and Exchangers I

715-Pos Board B495
Arrayed Lipid Membranes on Femtoliter Chambers Allow Highly Sensitive Detection of Ion Translocation Catalyzed by Transporter Protein
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Femtoliter reaction chamber array enables highly sensitive and quantitative biological assays such as single-molecule enzymatic assay, and digital PCR. Although femtoliter chamber arrays are very powerful for protein science as well as for biomedical applications, most of them have been in general limited to use for water-soluble proteins, due to the technical difficulties in preparing uniform and stable lipid bilayers. Here, we report an arrayed lipid bilayer chamber system (ALBiC) that displays a sub-million of femtoliter chambers, each equipped with micron-size electrodes, and sealed with a stable lipid

bilayer membrane with extremely high efficiency of over 90 %. When reconstituted an ion transporter, F_oF_1-ATP synthase (F_oF_1), into the bilayers of ALBiC, proton translocation driven by catalysis or membrane voltage were observed, showing that the highly sensitive detection of ion translocation is performed in ALBiC. Next, to explore the feasibility of single-molecule detection of transporter activity, we conducted the same assay in a condition where only a few molecules (0, 1, or 2 molecules) of F_oF_1 were reconstituted into each bilayer. The results showed that the response to proton translocation was no longer homogeneous between chambers, i.e. stochastic and quantized proton translocation was observed, demonstrating that the single molecule analysis of ion translocation catalyzed by transporter protein is first achieved in this study. Thus, the new platform, ALBiC, largely extended the versatility of femtoliter chamber arrays, and holds promise for understanding the working mechanism of transporter proteins as well as for further analytical and pharmacological applications.

716-Pos Board B496

Voltage Dependent Conformational Changes of the Na^+/K^+-ATPase Revealed by Site Directed Fluorometry

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Most of the voltage dependence of the Na^+/K^+-ATPase cycle originates from steps associated with extracellular Na^+ binding/release and occlusion/deocclusion transitions. In order to explore regions of the Na^+/K^+ pump that respond to voltage, we engineered single cysteine mutants facing the outside of the α and β subunits as targets of cysteine-reactive tetramethyl rhodamine (TMRM). These pumps were expressed in *Xenopus* oocytes and voltage-clamped to obtain simultaneous electrical and optical recordings. We have detected voltage dependent fluorescence changes when TMRM is conjugated at sites of the α and β subunits. Interestingly, positions within the β subunit produced robust voltage dependent fluorescence signals. These fluorescence changes follow the kinetics of Na^+ translocation through the pump. Quenching of the fluorescence signal can be altered by tryptophan mutations at the external side of the α subunit, indicating movement of the external face of the α subunit. Surprisingly, in some positions fluorescence changes persist even in the presence of ouabain, a specific inhibitor of the pump, and these changes are modified by tyrosine mutations in the transmembrane segment of the β subunit. These results suggest that there are intrinsic voltage-dependent conformational changes in the Na^+/K^+ pump. Supported by U54GM087519 and GM030376.

717-Pos Board B497

Na^+/K^+-ATPase Pumping Mechanism: Insights from Simulations

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The Na^+/K^+-ATPase resides in the plasma membrane and maintains the physiological K^+ and Na^+ concentration gradient across the cell membrane. It functions via a ping-pong mechanism, making iterative transitions between inward-facing (E1) and outward-facing (E2) conformations. The E1 conformation binds three Na^+ from the cytosol and exports them using the energy from ATP hydrolysis. The release of Na^+ and the binding of K^+ at the extracellular side trigger the structural transition to the E2(K2) state, which imports two K^+, followed by the pump returning to the E1 conformation. Although the broad features of the pumping cycle are known, the transition mechanisms between the conformational states and why a given state preferentially binds K^+ or Na^+, two monovalent cations of very similar radius, is not understood. Starting from the available x-ray structures of the Na^+/K^+-ATPase and the SERCA Ca^{2+}-pump, we use anisotropic network model pathway calculations and targeted molecular dynamics simulations to generate atomic models of the outward facing Na^+/K^+-ATPase, Na3E2-P and K2E2-P. Qualitative support of the models comes from a recent Na^+/K^+-ATPase x-ray structure and previous mutagenesis data. In addition to this, the gating charge and dissociation constants of the extracellular Na^+/K^+ binding calculated using the models are in excellent agreement with experimental data as well. The model generation process also produces the occlusion/de-occlusion transition pathways upon ion binding. To study the change in ion selectivity during the occlusion/de-occlusion process, free energy perturbation calculations are performed. The results reveal the molecular determinants of the 3Na-2K stoichiometry of the Na^+/K^+-ATPase.

718-Pos Board B498

Towards Thermodynamic Characterization of Transport Cycle in Secondary Transporters using Enhanced Sampling Techniques

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Secondary active transporters undergo large-scale conformational changes to couple the uphill and downhill transport of substrates. We have developed a powerful computational approach for the study of secondary active transport by combining several state-of-the-art enhanced sampling techniques primarily based on loosely coupled multiple-copy MD simulations (e.g., Bias-Exchange Umbrella Sampling and String Method with Swarms of Trajectories) within a novel, empirical, iterative sampling framework. Using this novel approach we were able to, for the first time, reconstruct an entire thermodynamic cycle associated with a secondary active transporter, namely, Glycerol-3-phosphate transporter (GlpT), based on its only available crystal structure. We have calculated the free energy profile of GlpT along a ''cyclic'' transition pathway connecting four distinct states of GlpT-phosphate complex including inward-facing apo, inward-facing bound, outward-facing bound, and outward-facing apo. Our results, which are in agreement with alternating access mechanism, indicate that the substrate binding lowers the free energy barrier of the transition between the inward- and ouward-facing states. When the substrate is present, the global conformational changes of the protein are coupled to the substrate translocation within the binding site. These results particularly highlight the significance of coupling between the local conformational changes of the binding site and global conformational changes of the protein. The simulations performed take advantage of tens to hundreds of loosely coupled all-atom MD simulations of GlpT in an explicit membrane/solvent environment with a total simulation time equivalent to ~20 microseconds of single-copy MD on a system of ~125,000 atoms. The novel approach developed here, which attempts to address the complexities associated with large-scale conformational changes of transporters and their coupling with the substrate translocation, may open opportunities for the study of other secondary transporters using enhanced sampling techniques and state-of-the-art supercomputing.

719-Pos Board B499

Protonation States of Key Acidic Residues at the Ion Binding Sites in Na/K Pump by QM Calculations

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Sodium-potassium ATPase transports three Na^+ ions outward and two K^+ ions inward by alternating two major conformational states, E1 and E2. There are three high affinity Na^+ binding sites in E1 and two K^+ binding sites in E2. These binding sites are located closely to each other in a transmembrane region containing several acidic residues. The protonation states of the acidic residues have been postulated to play an important role in ion selectivity. As the protonation states are strongly coupled, pKa estimations with programs, such as MCCE and PROPKA, using empirical point charges are ambiguous. It is questionable if conventional molecular dynamics simulations, though widely used, are applicable to determine the protonation states of strongly interacting residues, especially of protonated carboxyl groups. We therefore introduced quantum mechanical (QM) calculations in order to determine the positions of protons by calculating electrostatic potential (ESP) from electron densities. Starting with a model in which all acidic residues were deprotonated, the ESP showed several minima close to the carboxyl groups. The next model was generated by adding a proton at the minimum, and the ESP was calculated in the same way. Such procedure was repeated several times until no distinct minimum was found near the carboxyl groups. Finally, the geometry of the model was refined by QM to yield the model with explicit protons.

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Na^+/K^+ Pump Ion Binding Site Interactions Regulate the Proton Leak Pathway

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The Na^+/K^+ pump (NKA) is a membrane bound transporter located in animal cells which transports three Na^+ ions out for two K^+ ions into the cell for every one ATP molecule hydrolyzed. Two out-of-three ion-binding sites within the protein reciprocally bind two K^+ or two Na^+ ions (shared sites), while the remaining site exclusively binds Na^+. Without these two ions present the NKA passively transports H+, likely through the Na-exclusive site. In order to understand the proton transport mechanism voltage-clamp was used to study the